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# Severity of Footrot in Sheep through Experimental Infection with *Fusobacterium necrophorum* and *Dichelobacter nodosus*

*Research Article*

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## Abstract

Ovine footrot is an infectious dermatitis present over the world, causing economic losses, due to costs treatments and prophylaxis measures, as well as animal welfare concern. The etiology of footrot is complex involving a mixed bacterial infection modulated by environmental conditions. Footrot is caused by Dichelobacter nodosus, as the main etiological agent, while Fusobacterium necrophorum, a secondary pathogen in footrot is reportedly ubiquitous on pasture. In this experiment, we infected sheep by the two bacteria to investigate pathogenicity of footrot. Sheep were monitored over 4 weeks for clinical symptoms and presence of Fusobacterium necrophorum and Dichelobacter nodosus specific DNA by real time PCR. joint and abscess in interdigital area, 2 days post infection with high bacteria load. Sheep re-infected with Dichelobacter nodosus showed increase in symptoms severity.

**Keywords:** Sheep; Fusobacterium necrophorum; Dichelobacter nodosus; Experimental infection; PCR

**Abbreviations**: D. nodosus: Dichelobacter nodosus; F. necrophorum: Fusobacterium necrophorum; G: Group; DPI: Day Post Infection; PI: Post Infection; D: Day; CFU: Colony-Forming Units; Ct: Threshold Cycle; PCR: Polymerase Chain Reaction; SFR: Severe Footrot

## Introduction

Ovine footrot is a contagious foot disease of sheep with a global distribution [1,2]. The economic and welfare impact of the disease on sheep farming and affected animals respectively is considerable [3-5]. The clinical signs of footrot may vary from mild interdigital dermatitis (benign footrot), to separation of the sole and abaxial wall, resulting to complete separation of the hoof capsule [6,7]. The primary pathogen was first identified as Dichelobacter nodosus (D. nodosus), anaerobe bacteria with virulence factors involved in the pathogeneses of the disease, type IV fimbriae, extra-cellular serine proteases and produces a powerful enzyme that dissolves hoof horn and leads to severe lameness seen with classic virulent footrot [8-10]. A second pathogen Fusobacterium necrophorum (F. necrophorum), an opportunistic pathogen with reservoirs in healthy individuals causing necrobacilloses disease characterized by necrotic lesions and abscesses [11,12]. F. necrophorum, product several virulence factors such as leukotoxin, hemolysin and hemagglutinin, that play an essential role in the infection process and acts as a secondary invader in footrot and not as driver of the infection, even though its role as an initiator of infection with D. nodosus, or a synergistic action of both, has been suggested [6,13-15]. In sheep, F. necrophorum invades first the interdigital skin causing lesions and slight inflammation, the second stage of the disease is marked by the penetration of D. nodosus in the tissues witch responsible agent of aggravation of the lesions until the detachment of the horn [7,10]. Although other bacteria including spirochaetes have been suggest playing a role in footrot [16-19].

The identification of the molecular mechanisms in particular the paramount role of AprV2 in bacterial virulence, was a stepping stone in understanding factors that influence disease outcomes [20,21]. The etiology of ovine is complex involving infection by multiple bacterial species modulated by environmental conditions high temperatures or humidity [22-24]. In this study, we characterize strains of D. nodosus and F. necrophorum in culture and evaluated pathogenesis by experimental infection of sheep by the bacteria alone or in co-infection with D. nodosus to determine the role of each bacteria in the disease signs.

## Methods and Material

### Preparation of the Bacterial Suspension

Fusobacterium necrophorum: The used strain of F. necrophorum

subsp. necrophorum (ATCC 25286), was grown in anaerobic brain heart infusion broth (BHI; Difco) supplemented with vitamin K and hemin. A late-log phase culture (7 to 8h) with an absorbance of 2.4 at 600 nm, as measured by spectrophotometer, with a cell concentration of 108 CFU/mL, was centrifuged at 3500 rpm for 30 min at 4°C. The pellet of the culture was suspended in 10 ml PBS buffer and used to infect animals.

Dichelobacter nodosus: The challenge strain of D. nodosus (ATCC 31545) was grown in anaerobic brain heart infusion broth (BHI; Difco) supplemented with vitamin K and hemin at 37°C during 48 hours. Confluent growth of the bacteria was confirmed on 4% hoof agar plates. 80 ml of the whole culture containing 107 bacteria/ml was used to infect animals.

**Experimental Infection in Sheep**

Eight 6 to 9 months sheep of Sardi breed, weighing around 35 Kg, were supplied by a farm that had no history of footrot. Swabs were taken from the interdigital skin on the foot of all lambs, and tested for D. nodosus and F. necrophorum by PCR. Sheep were maintained in animal boxes (Biosecurity level 3 containment) two weeks under observation before starting the experiment and were fed a complete balanced diet and water ad libitum. Infection was carried out according to international guidelines described for the care and handling of experimental animals, chapter 7.8 of the OIE Terrestrial Animal Health Code and Directive 2010/63/UE of the European commission. The protocol was submitted and approved by the Internal Committee and quality assurance department. Before the start of the study, the selected lambs underwent a clinical examination by a veterinary. Animals were divided into 4 groups of two animals each, and groups of animals were completely separated from each other in ABSL3 facilities. On the first day of the study, two sheep of G1 and two sheep of G3 were inoculated by intradermal route between the hooves with 108 CFU of F. necrophorum bacteria suspensions in 1 ml (Figure 1). Inoculation concerned the four feet and was done using insulin needle after prior disinfection of the site. On day 8 of the study, two animals of G2 and the two animals of G3 were inoculated by the D. nodosus strain by bandage of the 4 limbs, by placing sterile compresses soaked by 20 ml of the strain during three days. G4 was kept as control throughout the study. Care was taken to prevent cross-contamination during handling and feeding and gloves were changed between every animal. All animals were observed daily, with rectal temperature and clinical scoring based on the severity of observed symptoms with a notation system from 0 to 3 or 4 for lameness as described in Table 1.

**Table 1**: Scoring system used to assess clinical signs after inoculation of Fusobacterium necrophorum and D. nodosus in sheep.

| Clinical Signs | | Score |
|---|---|---|
| Hyperthermia | Normal | (0) |
| | 40 < T°C < 40.5 | (2) |
| | 40.5<T°C<41 | (3) |
| | T°C > 41 | (4) |
| Lameness | Absence | (0) |
| | Light | (1) |
| | Moderate | (2) |
| | Severe | (3) |
| | Raised foot | (4) |
| Swelling of the joints | Absence | (0) |
| | Light | (1) |
| | Moderate | (2) |
| | Severe | (3) |
| Abscess | Absence | (0) |
| | Light | (1) |
| | Moderate | (2) |
| | Severe | (3) |
| Evolution of symptoms | One week | (1) |
| | Two weeks | (2) |
| | Three weeks | (3) |

**Figure 1:** Inoculation of Fusobacterium necrophorum strain via intradermal route between the hooves in sheep.

**Sampling and Treatment**

Swabs between the hooves were collected from sheep of Groups 1, 3 and 4 every 3 days post infection (dpi) from D0 (day) to D27 and for G2 every 3 days from D12 to D27 pi. Swabs were analyzed by quantitative real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) to monitor bacteria charge according.

Swabs samples were transported to the laboratory on ice and analyzed by PCR for bacteria genome detection. Swabs were collected in 2 ml PBS, homogenized and centrifuged at 2000 rpm for 20 min at 4°C. The supernatant was used for analysis.

**Quantitative Real-Time PCR**

All swabs were screened for bacterial genome detection by real time PCR. DNA extraction was carried out using Isolate II genomic DNA

kit (Bioline), and eluted in 100µl of elution buffer according to the manufacturer's instructions.

A quantitative real-time PCR (qPCR) TaqMan assay targeting the gyrB subunit gene [25], was used to determine the load of F. necrophorum DNA in samples. The strain ATCC was used as positive control.

A quantitative real-time PCR (qPCR) TaqMan assay targeting aprV2 and aprB2 proteases genes [17], respectively detected in virulent and benign strains of D. nodosus [21], was used to determine bacterial genetic loads and differentiation of virulent and non-virulent strains in clinical samples using the aprV2 and aprB2 protease genes [21].

## Results

### Hyperthermia

Sheep inoculated at D0 with F. necrophorum strain (G1 and 3), showed hyperthermia above 39.5°C from the second day until D8 pi, with the peak at D3 (40.4°C) (Figure 2). For G2, sheep infected with D. nodosus strain at D8 developed early hyperthermia the first dpi with a peak 40.6°C at D13, the temperature remained up to 40°C during 8 days for animal 1 and 11 days for animal 2. In G3 infected with the two bacteria at D0 and D8, hyperthermia was recorded 14 days following the 2nd infection with D. nodosus. Average temperature of control animals (G4) remained normal throughout the experiment.

### Clinical Symptoms and Scoring

Animals inoculated with F. necrophorum developed lameness in posterior limbs starting D2 pi. Inflammation and swelling in interdigital area of hind limbs was observed on D4 in all infected sheep with redness and warm foot evolving to fissure with scabby exudate in the margin. On D8, the foot of the left posterior member was lifted and when moving the head is tilted (Figure 3). Development of abscess at the site of inoculation in posterior limbs and swelling was maximal at D9 or D10 pi. The abscess burst and discharged cream or pale green caseous pus with fool odor. At D12 pi, abscesses generalized in the 4 members of the two inoculated sheep (Figure 3). The same clinical symptoms and evolution was observed in G1 inoculated with F. necrophorum and G3 inoculated with F. necrophorum following with D. nodosus. However, severity of lesions was the toughest in G3.

Animals infected with D. nodosus strain (G2) developed a lameness for 7 days starting D4 pi. Swelling of the joints and redness of interdigital areas were noted from D6 pi in limbs of the two inoculated sheep for one week. No abscess was registered on the hooves of the two sheep.

Animals of G1 and G3 recovered in 3 weeks and eleven days pi in G2. Control animals (G4) have not developed any clinical symptoms and remained in good conditions. Clinical scoring after infection with F. necrophorum was 13, after infection with D. nodosus 7 and with both 15 in G3 (Table 2).

PCR Results

The bacteria genome of F. necrophorum has been detected by PCR up to D21 in G1 and up to D27 pi in G3 with a maximal Ct of 21,5 and 18 respectively at D18 pi (Table 3). For G2 inoculated with D. nodosus, PCR was positive up to D7 pi with D. nodosus with a maximal Ct of 31.6. In G3, animals were tested positive with a Ct 35.9 at D4 pi with D. nodosus (Table 3). Differentiation between virulent and benign strains of D. nodosus by PCR based on aprV2 and aprB2 protease genes, showed that the used ATCC strain is virulent.

**Table 2:** Clinical scoring observed in sheep infected with F. necrophorum and D. nodosus.

| Infection | T° | Lameness | Swelling | Abscess | Evolution | Total Scoring |
|---|---|---|---|---|---|---|
| F. necrophorum | 2,25 | 3,25 | 2,5 | 2 | 3 | 13 |
| D. nodosus | 2,5 | 1,5 | 2 | 0 | 1 | 7 |
| F. necrophorum and D. nodosus | 2 | 4 | 3 | 3 | 3 | 15 |

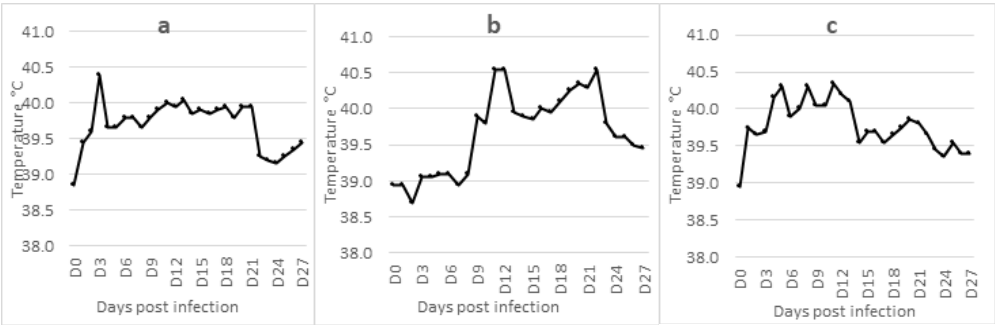


**Figure 2**: Average temperature post infection: a: animals infected with F. necrophorum; b: animals infected with D. nodosus; c: animals infected with F. necrophorum and D. nodosus.

**Table 3:** Clinical scoring and bacteria charge observed in sheep infected with F. necrophorum and D. nodosus.

| Infection | PCR | | Clinical Scoring |
|---|---|---|---|
| | Ct | Duration (days) | |
| F. necrophorum | 21.5 | 21 | 13 |
| D. nodosus | 31.6 | 7 | 7 |
| F. necrophorum | 18 | 27 | 15 |
| and D. nodosus | 35.9 | 4 | |

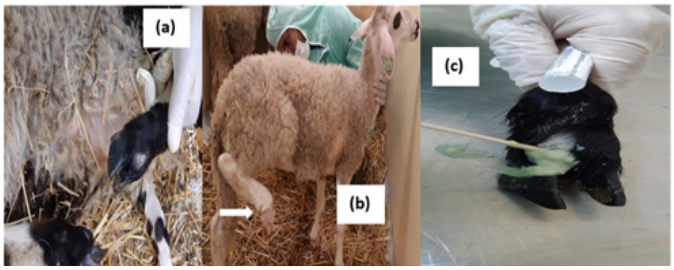


**Figure 3**: Signs observed on infected sheep. Swelling on previous members (a), raised foot (b) and abscess inside the hooves (c).

## Discussion

The pathogenesis of footrot is very complex and multifactorial [4,26]. Footrot is a mixed bacterial infection, in most cases F. necrophorum is in all sheep and goat environments and sets a primary stage of infection allowing footrot to occur with D. nodosus surinfection [6,27].

Maboni et al. [28] and Witcomb et al. [14] reported that D. nodosus and F. necrophorum are almost exclusively observed in all stages of epidermis disease [7,28]. The role of each bacteria in the pathogenesis of footrot steel controversial. In a study examining load of D. nodosus and F. necrophorum during natural infection, Witcomb et al. [14] found an increase in load of D. nodosus before and during an episode of interdigital dermatitis and prior to occurrence of severe footrot (SFR) [14]. In contrast, the F. necrophorum load increased only when SFR had occurred. The authors concluded that D. nodosus initiates disease and F. necrophorum is an opportunist once disease has occurred. Other study reported that F. necrophorum is thought to have a role in pathogenesis particularly as an initiator of damage to the interdigital skin thereby allowing entry of D. nodosus [6,29].

In order to determine the impact of the primary infection by F. necrophorum on the apparition of disease symptoms, sheep were infected with a virulent strain and observed clinical symptoms with or without re-infection with D. nodosus. Evaluation was based on clinical scoring and bacteria charge obtained on sensitive sheep after experimental infection. Despite the fact that footrot exist in all domestic ruminants, sheep is the most affected by the disease because of frequent movements specially in extensive breeding which is dominant in Africa and Asia. Indeed symptoms reported in sheep are well described and reported to be more severe than in goats or cattle [30,31].

In 1941, Beveridge identified D. nodosus as causal agent. When sheep feet were inoculated with D. nodosus, footrot developed, however, when sheep were inoculated with F. necrophorum, lesions did not resemble to footrot. Beveridge concluded that F. necrophorum was likely to be a secondary invader in footrot increasing lesions severity [28]. In our knowledge, there is no report in of coinfection with F. necrophorum and D. nodosus to reproduce footrot disease in sheep. In addition, groups of animals inoculated by only one strain (D. nodosus or F. necrophorum) was used which essential if the model was to be used in the assessment of vaccines efficacy. In this study, we did not succeed to reproduce typical lesions of footrot in sheep using D. nodosus alone or in association with F. necrophorum.

Infected sheep with F. necrophorum showed symptoms characterized by fever, lameness, raised foot and abscess with pus in interdigital area. Used route of infection is the most appropriate reproducing natural conditions and allowed to secure the quantity of administered bacteria. The disease evolves during three weeks with characteristic symptoms and lesions. The injected dose of F. necrophorum seems to be sufficient to induce typical symptoms. Corner et al. [32] successfully produced lesions in sheep by inoculation F. necrophorum into skin devitalized by freezing. All feet that were inoculated with 5.108 developed marked swellings by D2 to 4 pi, there were trends in the data showing increasing severity with increasing dose [32].

Recently, strains of D. nodosus have been shown to exist in two distinct forms: virulent or benign, based on the type of disease caused under optimal climatic conditions and our differentiation PCR confirmed the virulent pathogenicity [5,10]. In this study, sheep infected with D. nodosus showed mild symptoms such hyperthermia, lameness and swilling and co-infection with both bacteria increased severity of the primary infection with F necrophorum. Bennett et al. 2009 reported that D. nodosus is unable to reproduce symptoms of this disease on its own, and F. necrophorum is indispensable to induce footrot in sheep [33].

## Conclusion

In this study, Fusobacterium necrophorum induces typical signs of ovine interdigital dermatitis, and enhances the severity of footrot, but may not be essential to the disease process in all animals, and cannot induce the disease alone.

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## Conflict of Interests

The authors declare that they have no competing interests.

## References

1. Goddard P, Waterhouse T, Dwyer C, Stott A (2006) The perception of the welfare of sheep in extensive systems. Small Rumin Res 62(3): 215–225.
2. Wani SA, Samanta I (2006) Current understanding of the aetiology and laboratory diagnosis of footrot. Vet J 171: 421–428.
3. Nieuwhof GJ, Conington J, Bunger L, Haresign W, Bishop SC (2008) Genetic and phenotypic aspects of foot lesion scores in sheep of different breeds and ages. Animal 2(9): 1289–1296.
4. Wassink GJ, George TRN, Kaler J, Green LE (2010) Footrot and interdigital dermatitis in sheep: Farmer satisfaction with current management, their ideal management and sources used to adopt new strategies. Prev Vet Med 96: 65–73.
5. Zanolari P, Dürr S, Jores J, Steiner A, Kuhnert P (2021) Ovine footrot: A review of current knowledge. Vet J 271: 105647.
6. Roberts D, Egerton J (1969) The aetiology and pathogenesis of ovine foot-rot: II. The pathogenic association of Fusiformis nodosus and F. necrophorus. J Comp Pathol 79(2): 217–227.
7. Witcomb LA, Green LE, Calvo-Bado L, Russell CL, Smith EM, et al. (2015) First study of pathogen load and localisation of ovine footrot using fluorescence in situ hybridisation (FISH). Vet Microbiol 176: 321–327.
8. Beveridge WIB (1941) Foot-rot in sheep: a transmissable disease due to infection with Fusiformis nodosus (n. sp.): studies on its cause, epidemiology and control. CSIRO Austr Bull 140: 1–56.
9. Dhungyel O, Schiller N, Whittington R (2015) Identification and characterization of serogroup M Dichelobacter nodosus from sheep with virulent footrot. Vet Microbiol 176(3-4): 378–381.
10. Kennan R, Han X, Porter C, Rood JI (2011) The pathogenesis of ovine footrot. Vet Microbiol 153(1-2): 59–66.
11. Frosth S, König U, Nyman AK, Pringle M, Aspán A (2015a) Characterisation of Dichelobacter nodosus and detection of Fusobacterium necrophorum and Treponema spp. in sheep with different clinical manifestations of footrot. Vet Microbiol 179(1-2): 82–90.
12. Raadsma HW, Dhungyel OP (2013) A review of footrot in sheep: new approaches for control of virulent footrot. Livest Sci 156: 115–125.

13. Clifton R, Green L (2016) Pathogenesis of ovine footrot disease: a complex picture. Vet Rec 225–227.

14. Witcomb L, Green L, Kaler J, Ul-Hassan A, Calvo-Bado L, et al. (2014) A longitudinal study of the role of Dichelobacter nodosus and Fusobacterium necrophorum load in initiation and severity of footrot in sheep. Prev Vet Med 115(1-2): 48–55.

15. Zhou H, Bennett G, Hickford JG (2009) Variation in Fusobacterium necrophorum strains present on the hooves of footrot infected sheep, goats and cattle. Vet Microbiol 135(3-4): 363–367.

16. Brugère-Picoux J (2004) Maladies des moutons. France agricole.

17. Frosth S, Ulrika K, Nyman AK, Pringle M, Aspan A (2015b) Characterisation of Dichelobacter nodosus and detection of Fusobacterium necrophorum and Treponema spp. in sheep with different clinical manifestations of footrot. Vet Microbiol 179: 82–90.

18. Gelasakis A, Kalogianni A, Bossis I (2019) Aetiology, risk factors, diagnosis and control of foot-related lameness in dairy sheep. Animals 9(8): 509.

19. Maboni G, Blanchard A, Frosth S, Stewart C, Emes R, et al. (2017) A distinct bacterial dysbiosis associated skin inflammation in ovine footrot. Sci Rep 7.

20. Kennan RM, Gilhuus M, Frosth S, Seemann T, Dhungyel OP, et al. (2014) Genomic Evidence for a Globally Distributed, Bimodal Population in the Ovine Footrot Pathogen Dichelobacter nodosus. American Society for Microbiology 5(5): 1–11.

21. Stäuble A, Steiner A, Normand L, Kuhnert P, Frey J (2014) Molecular genetic analysis of Dichelobacter nodosus proteases AprV2/B2, AprV5/B5 and BprV/B in clinical material from European sheep flocks. Vet Microbiol 168(1): 177–184.

22. Bennett GN, Hickford JGH (2011) Ovine footrot : New approaches to an old disease. Vet Microbiol 148(1): 1–7.

23. Mucha S, Bünger L, Conington J (2015) Genome-wide association study of footrot in Texel sheep. Genet Sel Evol 47(35).

24. Zhou H, Bennett G, Buller N, Hickford JGH (2011) Isolation of two novel Fusobacterium necrophorum variants from sheep in Australia. Vet Microbiol 148(2-4): 448.

25. Jensen A, Kristensen LH, Prag J (2007) Detection of Fusobacterium necrophorum subsp. funduliforme in tonsillitis in young adults by real-time PCR. Clin Microbiol Infect 13(7): 695–701.

26. Green L, George TR (2008) Assessment of current knowledge of footrot in sheep with particular reference to Dichelobacter nodosus and implications for elimination or control strategies for sheep in Great Britain. Vet J 175(2): 173–180.

27. Ozgen EK, Cengiz S, Uhican M, Okumus Z, Kortel A, et al. (2015) Isolation and identification of Dichelobacter nodosus and Fusobacterium necrophorum using the polymerase chain reaction method in sheep with footrot. Acta Vet Brno 84(2): 97–104.

28. Maboni G, Frosth S, Aspan A, Totemeyer S (2016) Ovine footrot: new insights into bacterial colonisation. Vet Rec 79(9): 228.

29. Parsonson I, Egerton J, Roberts D (1967) Ovine interdigital dermatitis. J Comp Pathol 77: 309–313.

30. Kaler J, Green L (2009) Farmers' practices and factors associated with the prevalence of all lameness and lameness attributed to interdigital dermatitis and footrot in sheep flocks in England in 2004. Prev Vet Med 92(1-2): 52–59.

31. Atia J, Monaghan E, Kaler J, Purdy K, Green L, (2017) Mathematical modeling of ovine footrot in the UK: the effect of Dichelobacter nodosus and Fusobacterium necrophorum on the disease dynamics. Epidemics 21: 13–20.

32. Corner LA, Collins ND, Vaughan JA (1996) An experimental ovine foot abscess model using a Fusobacterium necrophorum biotype AB. Vet Microbiol 48(1-): 1–7.

33. Bennett G, Hickford J, Sedcole R, Zhou H (2009) Dichelobacter nodosus, Fusobacterium necrophorum and the epidemiology of footrot. Anaerobe 15(4): 173-176.